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THE INACTIVATION OF VIRUSES IN WATER SUPPLIES BY ULTRA-VIOLET IRRADIATION

DIVISION OF RESEARCH

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INTRODUCTION

There is a great deal of evidence available to indicate that viruses, predominantly those of human intestinal origin, are present in surface waters. Sewage effluents, which may contain as many as 7-70 viruses per litre (1, 2), are probably the major contributors to this contamination, but viruses of animal and plant origin may also reach such waters by urban and rural run-off (3); the demonstration of these viruses is extremely difficult, but they have been detected on several occasions (4, 5). Where these waters are used for a potable supply, there is the possibility that viruses may enter the distribution system.

Chlorination has been used almost exclusively for the disinfection of all water supplies, but unless the treatment afforded polluted, surface waters is that of breakpoint chlorination, there is a real danger that viruses may survive the treatment process, as present methods involving combined chlorine may be inadequate (6). Under situations where facilities for stringent chlorination are not available, or other considerations preclude its use, alternative methods of treatment must be considered.

The lethal effect of ultraviolet (UV) light for microorganisms has been known for many years. UV irradiation is employed extensively for the inactivation of airborne microorganisms in hospital operating rooms and nurseries and is also used for the disinfection of water used in the preparation of food and drugs, where chemical forms of treatment are inappropriate. Advances in technology have led to the development of equipment which makes possible the application of UV irradiation in the treatment of water supplies, particularly those of relatively small capacity. With UV treatment thus an economic alternative to chlorination, an investigation into the effect of such radiation on viruses in water was warranted.

MATERIALS AND METHODS

The test organism used throughout this investigation was a bacteriophage of E.coli B. In many characteristics, bacteriophages or bacterial viruses resemble the animal viruses; however, they are technically simpler to use, and have proven to be useful tools in studies such as these. For example, Shuval (7) showed that Echovirus in sewage is inactivated at lower levels, poliovirus at slightly higher levels of chlorination than a bacteriophage.

The virus used in this study was prepared from a pure stock laboratory culture, and could be diluted to form a virus suspension containing the required number of virus particles. At intervals during the exposure period, samples were removed and the number of viruses surviving was determined. Assay was carried out using a most probably number (MPN) method (8). Where large numbers of viruses were expected, 1 ml of the treated suspension (or 1 ml of an appropriate dilution of it) was added to 100 ml of sterile tap water, which was then assayed for virus content; the amount of virus estimated by the method to be present in the 100 ml of water, was thus present in the 1 ml of suspension which had been added. Where low numbers of viruses remained, the treated

suspension itself was used in the assay and results could be expressed as virus per 100 ml of the original suspension. Although the MPN method yields only an estimate of the actual number of viruses present, it is particularly applicable where assay of low numbers of virus is required; the usual plaquing method (9), enables only a maximum of 1 ml of the sample to be tested. In these experiments, where few survivors remained, the five 1 ml samples taken for the MPN method were negative for virus, whereas the five 10 ml samples showed virus to be present; if only 1 ml samples were tested, destruction of virus would have been erroneously regarded as complete.

The efficiency of UV irradiation in the destruction of the bacteriophage was determined in a series of laboratory tests, where irradiation conditions could be controlled and also in experiments using an actual production model of an UV water sterilizer.

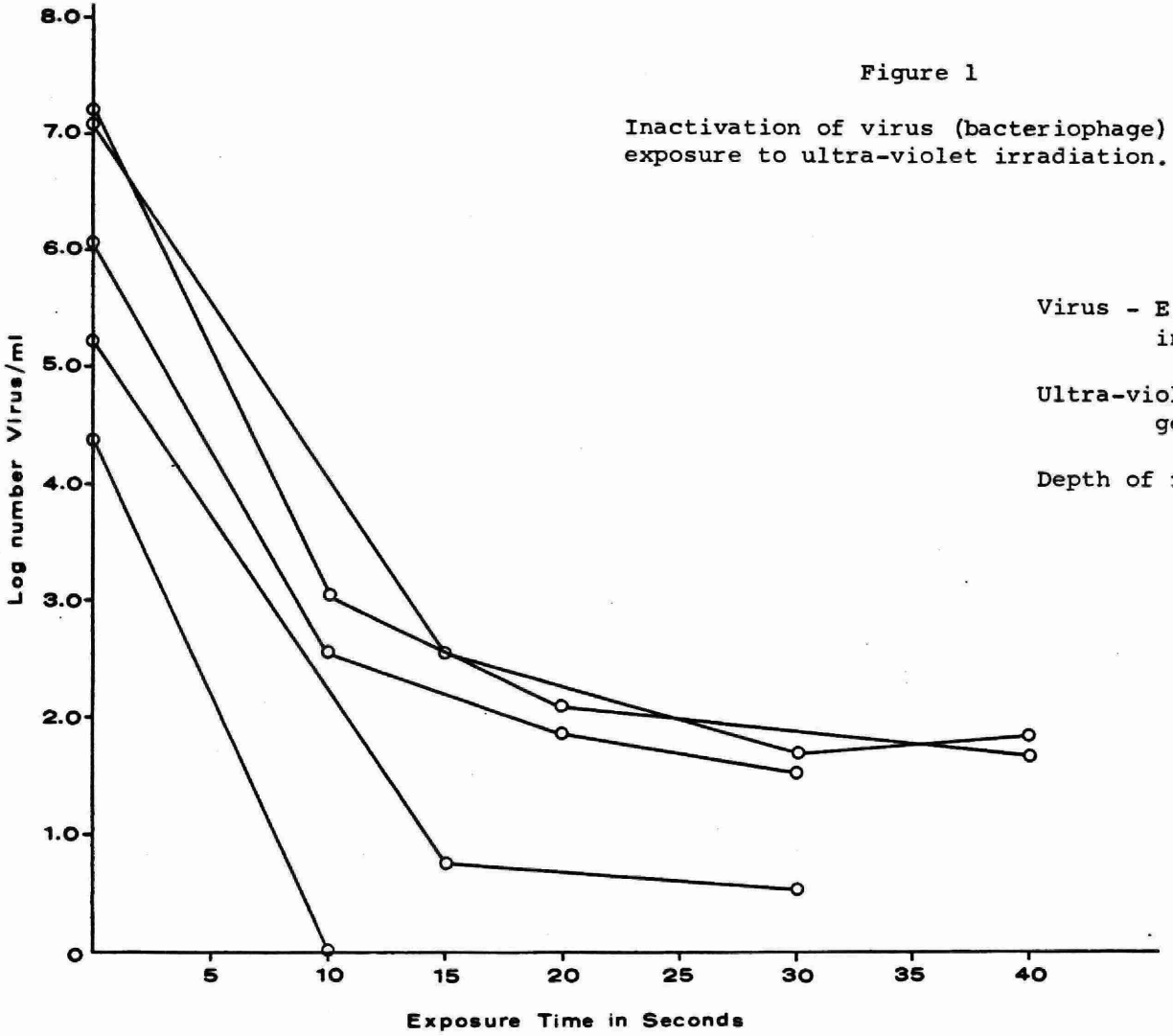
Laboratory Experiments

A virus suspension in tap water was placed in a small beaker and subjected to radiation from a GE germicidal tube (30 watt); the beaker was positioned such that the fluid surface was almost in contact with the tube, and the depth of suspension exposed was approximately one inch. Surviving

viruses were enumerated after irradiation periods of 10, 15, 20, 30 and 40 seconds. The inactivation curves obtained in a series of experiments in which different initial levels of virus were employed are shown in Figure 1. An exposure time of 10 seconds was required to reduce approximately 10^4 virus particles per ml to a non-detectable level; a 99.99% reduction or greater, was obtained at the end of a thirty second exposure period with initial levels of virus up to approximately 10^7 particles per ml. However, this reduction actually occurred after only 15 to 20 seconds of irradiation, with little further decrease even after 40 seconds. This phenomenon, which occurs with higher virus concentrations, has been noted by Huff and co-workers (10) with poliovirus; here, UV irradiation was found to completely inactivate the virus only up to levels of 10^3 to 10^4 per ml. The reason was thought to be a clumping of the virus particles at high concentrations, when the outer particles protect those inside; these therefore survive the effects of radiation, which has poor powers of penetration. However, even wastewaters would be expected to contain a total of virus particles of less than 10^4 per ml, which level is below that at which the above phenomenon apparently occurs.

Figure 1

Inactivation of virus (bacteriophage) upon exposure to ultra-violet irradiation.



Virus - E.coli B bacteriophage
in tap water

Ultra-violet source - GE
germicidal lamp, 30 watt

Depth of fluid exposed = 1"

Water Sterilizer Experiments

A schematic diagram of the commercial UV sterilizer* is presented in Figure 2; the apparatus consisted of the actual sterilizer unit and an activated charcoal filter for prefiltration. The filter was incorporated to remove particulate matter from influent water, which might otherwise reduce the efficiency of the process. In the sterilizing unit, the conditions for disinfection are essentially the same as those employed for the laboratory experiments. Thus, a 3-inch diameter glass tube, about 2½ feet long, has a 1-inch diameter quartz tube surrounding a 36 watt UV tube at its centre. The influent water, after passing through the pre-filter, flows between the inner and outer tubes, thereby exposing a 1-inch depth of fluid to the irradiation. Actual retention time in the unit, corresponding to the time for which the water is exposed to the irradiation, may be varied by adjusting the flow rate from about 11 seconds at 4 gallons per minute (gmp), to about 43 seconds at 1 gpm. The unit is provided with an automatic shut-off switch which is placed external to the unit and measures the intensity of UV light passing through the water; should this intensity fall below minimum prescribed limits, due to increased colour or turbidity change in the influent

* Aquacare water sterilizer

SCHEMATIC DIAGRAM of UV WATER STERILISER

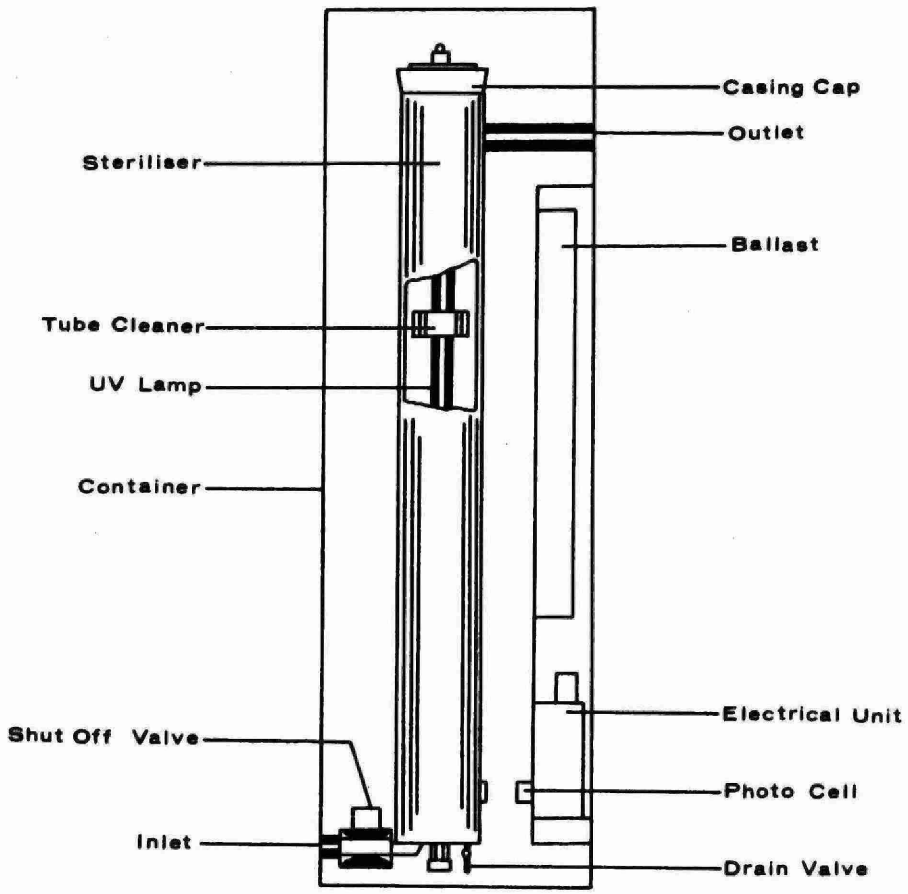


Figure 2

water or other causes, flow of water through the unit is prevented. This unit is fitted with a cleaning device, a collar which moves up and down the tube with fluctuations in water flow, thereby removing the build-up of debris which tends to occur on the surface of the quartz tube and would otherwise reduce the efficiency of radiation.

A total of 500 gallons of tap water was seeded to contain 2×10^4 viruses per ml and held in a large tank. This seeded water was run through the unit at varying flow rates, the effluent being assayed for virus content. To determine the effects of colour on disinfection efficiency, the seeded water was coloured to a level of approximately 100 Hazen units by the addition of brewed tea; the test procedure was then repeated. The results of two such experiments are shown in Table 1; in both, only at the slowest flow rate used, i.e. 1.5 gpm, corresponding to a retention time of 29 seconds, was complete inactivation achieved. Low levels of virus were detected at 3 gpm (retention time 14.5 seconds). A slight decrease of efficiency was noted when the water to be treated was coloured, but it should be pointed out that at this level of colour the automatic shut-off on the unit was activated.

Table 1

The Inactivation of Virus (Bacteriophage) on the Passage of Seeded Tap Water Through an Ultra-Violet Water Treatment Unit.

	Most probable number of virus per 100 ml water	
	Experiment 1	Experiment 2
Influent water*	2×10^6	2×10^6
Effluent water 5 gpm	23	8
Effluent water 3 gpm	2	2
Effluent water 1.5 gpm	No virus detected	No virus detected
Influent water**	2×10^6	2×10^6
Effluent water 5 gpm	46	79
Effluent water 3 gpm	5	5
Effluent water 1.5 gpm	No virus detected	No virus detected

* Tap water, seeded with virus, with a turbidity of 6.5 units.

** Tap water, seeded with virus and coloured to approximately 100 Hazen units by addition of brewed tea; turbidity 6.5 units.

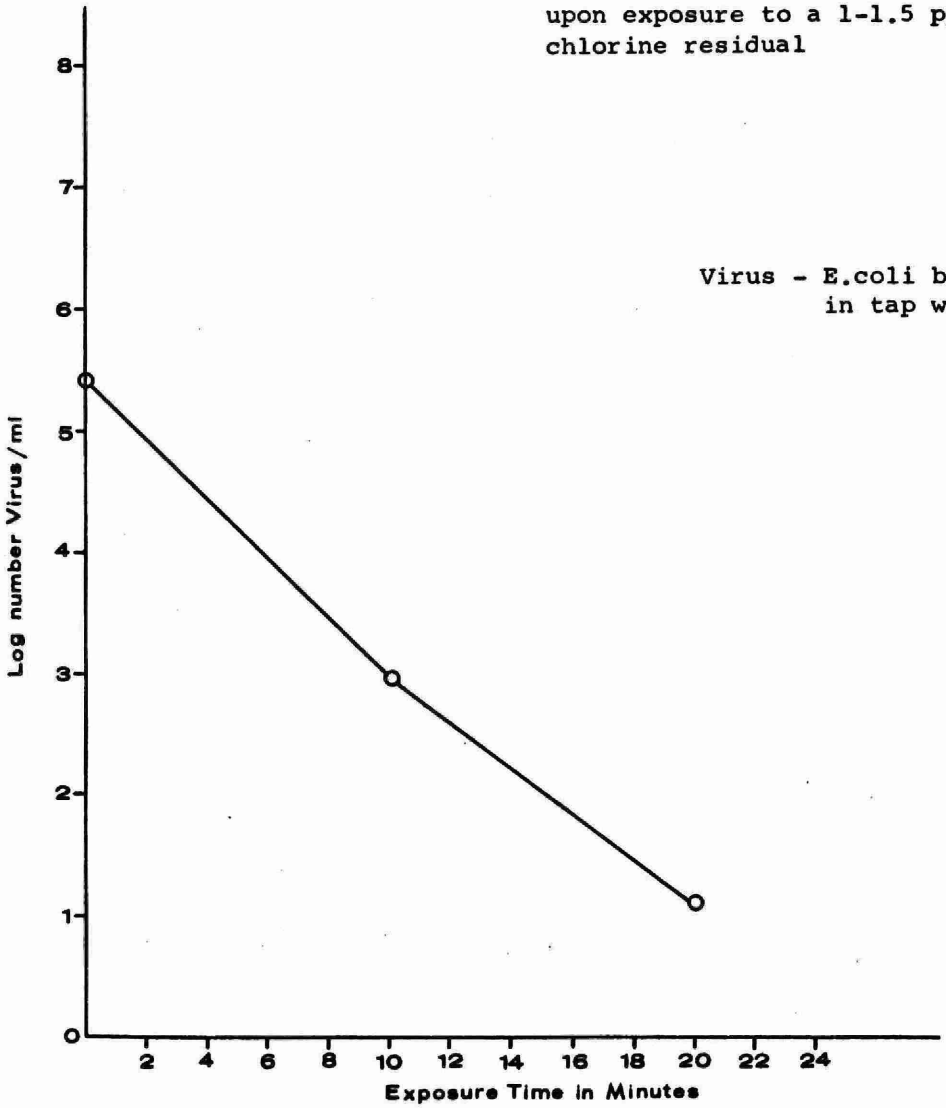
In one further test, polluted river water was passed through the unit at 6.5 gpm (retention time of 6.7 seconds), without prefiltration, and no virus was detectable in the effluent samples. This water contained about 100-200 viruses per 100 ml initially, and possessed a turbidity of 38 units (38 ppm SiO_2).

In order to create a basis for comparison, aqueous virus suspensions, similar to those used in the radiation experiments were exposed to a free chlorine residual (measured by the OTA method) of 1.0 to 1.5 ppm. Chlorine effect was neutralized by dilution, upon removal of the 1 ml samples prior to assay. The inactivation obtained is shown graphically in Figure 3, where an exposure of about 20 minutes is required to produce a little more than a 99.99% reduction of virus numbers. This agrees fairly well with the figures given by Chang (11), for poliovirus, Coxsackie A2 and A9 in waters of pH below 8.0 and temperatures above $4^{\circ}C$.

Figure 3

Inactivation of virus (bacteriophage) upon exposure to a 1-1.5 ppm free chlorine residual

Virus - E.coli bacteriophage in tap water



DISCUSSION AND CONCLUSIONS

The results indicate that UV treatment of water from the point of view of virus inactivation, is worthy of consideration. Under the conditions of the tests, UV treatment would appear to be capable of producing a virus-free effluent, when viruses are present in the raw water at levels at which they would be expected to occur in polluted surface waters. Where initial virus levels are in excess of 10^4 per ml, UV irradiation will not produce a sterile effluent, even on prolonged exposure. An apparent discrepancy occurs between the laboratory experiments and those performed with the commercial unit; inactivation of approximately 10^4 viruses per ml occurred in about 10 seconds under the former conditions, but not under the latter. This may be explained simply as being caused by mixing effects or by either or both of the following reasons; the intensity of radiation to which the suspensions were subjected may not have been exactly identical in the two sets of experiments, and no means were available. to determine this. Theoretically, however, the 30 watt bulb used in laboratory tests should produce a lower intensity dose of UV radiation than the 36 watt bulb in the unit. Additionally, in the laboratory tests, only 1 ml samples of

treated virus suspensions were tested, since virus levels were high; in the experiments with the unit, a total of 55.5 ml of treated water was tested, and 1 ml samples of such effluent water showed the absence of viruses. It is apparent that some caution must be exercised in the extrapolation of laboratory procedures to the field. Techniques which have worked well under rigorously controlled laboratory conditions occasionally demonstrate decreased efficiency upon field application.

With respect to comparison with the anti-viral activity of chlorine, it should be emphasized that the reductions refer to those obtained with free chlorine residuals, and water with a high ammonia or organic content would probably yield an unacceptable water under such circumstances, due to too high a combined chlorine content. It is under these conditions, or those where normal chlorination practices lead to the production of taste and odour problems, that UV treatment may prove an adequate substitute.

There are several disadvantages to UV treatment immediately obvious. There is no residual of any kind remaining in the treated water to protect supplies in the distribution system from subsequent contamination; however, the normal chlorine residual remaining would also offer little protection

against such contamination. The initial equipment costs, maintenance and operating expenses are greater than for chlorination under most circumstances, although the installations are largely automatic and require little adjustment after initial set-up. Since the disinfection process depends upon the transmission of the UV light through the water, the equipment should probably only be considered where the raw water source is of fairly good quality and relatively free of colour, turbidity and metal ions.

There is no means of determining if the water has been properly treated; reliance must be placed on the automatic shut-off in preventing the release of water which has not been adequately irradiated.

There are also several advantages to this form of treatment, in that there is no danger of overtreatment and taste and odour problems are not compounded by the addition of chemical substances.

Although it has been used successfully in the virological disinfection of sea water in connection with the shellfish industry (12), final proof of the efficacy of UV treatment in the inactivation of viruses in water supplies probably awaits further field trials using the actual enteric viruses.

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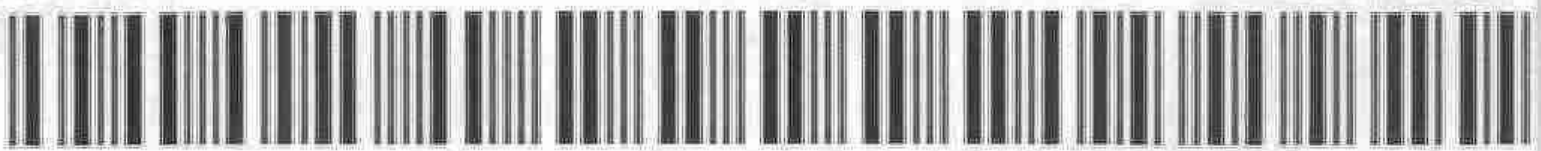
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